PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/002726	17.03.2004	27.03.2003

International Patent Classification (IPC) or both national classification and IPC
C12P7/22

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Fausti, S

Telephone No. +49 89 2399-7389

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/002726

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 13 (partially)

because:

☒ the said international application, or the said claims Nos. 13 (partially) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	-
	No:	Claims	1-14
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-12, 13(partially), 14
	No:	Claims	-

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III.

N.1 Claim 13, in so far as it concerns diagnosis and analysis, can relate to a diagnostic method practised on the human or animal body. Such a method relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of claim 13 in so far as it concerns diagnosis and analysis (Article 34(4)(a)(I) PCT).

Re Item V.

1 DOCUMENTS.

The following documents are referred to in this communication:

D1 : Liese A. et Al., *Journal of Molecular Catalysis B: Enzymatic* (1998) Vol. 4, Pages 91-99;
D2 : Jonsson A. et Al., *Biochimica Et Biophysica Acta. Protein Structure And Molecular Enzymology* (1999) Vol. 1430, No. 2, Pages 313-322;
D3 : Groeger H. et Al., *Organic Letters* (2003) Vol. 5, No. 2, Pages 173-176;
D4 : De Carvalho C.C.C.R. et Al., *Journal of Molecular Catalysis B: Enzymatic* (2002) Vol. 19-20, Pages 389-398;
D5 : Schmid A. et Al., *Journal of Molecular Catalysis B: Enzymatic* (2001) Vol. 11, No. 4-6, Pages 455-462;
D6 : Kato T. et Al., *Analytical Biochemistry* (1973) Vol. 53, No. 1, Pages 86-97;
D7 : Kato T. et Al., *Analytical Biochemistry* (1982) Vol. 126, No. 1, Pages 44-51;
D8 : US6242234.

1.1 D1 discloses the enantioselective reduction of poorly water-soluble ketones by means of alcohol dehydrogenase in an emulsion membrane reactor with in-situ cofactor regeneration by formate dehydrogenase (see abstract and figures 1-2). The pure substrate is 1% of the whole reactor volume and is emulsified with the aqueous solution, which is filtered from the emulsion and circulated through the enzyme reactor (see the paragraph joining pages 93 and 94).

1.2 D2 discloses the enantioselective reduction of ketones by means of alcohol

dehydrogenase with in-situ cofactor regeneration by an enzyme co-substrate (see abstract and the first paragraph of the left-hand column on page 315). Different reaction media like organic solutions of n-hexane with increasing water content, biphasic water/n-hexane systems and aqueous solutions have been tested: increasing amounts of water improve the enzymatic conversion of the ketone substrate (see abstract). In a specific embodiment, the substrate is not completely soluble in the aqueous medium at the overall concentration of 50 mM (see table 2).

1.3 D3, D4 and D5 disclose the stereoselective enzymatic transformation of organic substrates by means of cofactor-dependent enzymes with in-situ cofactor regeneration (see: D3, abstract and scheme 2; D4, abstract; D5, abstract). The preferred reaction media are biphasic systems comprising water and an organic solvent, the latter representing a reservoir for poorly water-soluble substrates and an extraction medium for the products (see: D3, tables 1-2 and the second paragraph of the left-hand column on page 175; D4, page 391; right-hand column, last paragraph; D5, table 1). In addition, D4 discloses pure organic or pure aqueous reaction media; the aqueous reaction medium contains a limited amount of the partially immiscible substrate (see table 1).

1.6 D6 and D7 disclose diagnostic and analytical methods based on the enzymatic amplification of the NAD cofactor (NAD cycling) (see abstracts).

1.8 D8 discloses methods for the enzymatic preparation of aminoacids by means of leucine dehydrogenase, which is "coupled" with formate dehydrogenase for the purpose of regenerating the cofactor required by the first enzyme (see scheme 1). According to these methods, the keto-acid substrate is completely solubilized in the reaction medium (see examples 6 and 7).

2. CLARITY (Art. 6 PCT).
(Issues relevant for the interpretation of the claims)

2.1 The feature of the minimum substrate amount, which exceeds the solubility of the substrate in the aqueous solution as defined in claim 1, relates to a method of using the claimed reaction system rather than clearly defining this system in terms of its technical features (see "the substrate is employed... in an amount of..."). The intended limitations are therefore not clear, contrary to the requirements of Article 6 PCT.

2.1[a] The claimed subject-matter would not be novel (Art. 33(2) PCT) over the prior art, if this unclear feature is completely neglected. For example, the coupled enzymatic reaction systems comprising aqueous reaction media, which are disclosed in D4 and D8 (see points 1.3 and 1.8 above), would fall within the claimed scope under this hypothesis.

2.1[b] For examination purpose, the claim has been therefore interpreted as it would define a coupled enzymatic reaction system containing such a minimum amount of the substrate.

2.1[c] The same observation applies to the surfactant feature (see "without addition..."), which is interpreted as referring to the absence of any surfactant.

2.2 In addition, the expression "purely aqueous solvent system" of claim 1 is not clear in the context of the claims and the accompanying examples because, in certain embodiments (like the preferred ones), the claimed reaction system involves a biphasic system comprising an organic liquid phase, i.e. the organic substrate exceeding the solubility limit. This organic liquid is to be considered "a priori" a solvent that, for example, contains a minimum amount of water dissolved therein. Hence, no "purely aqueous solvent system" according to a strict interpretation of this expression is involved, but an aqueous/organic biphasic solvent system is to be considered for the claimed reaction system.

2.2[a] Nevertheless, a broad interpretation that would include any aqueous/organic biphasic solvent system does not appear to be meaningful. Under such a broad interpretation, the claimed subject-matter is clearly not novel (Art. 33(2) PCT) over the coupled enzymatic reaction systems with biphasic reaction media disclosed in the prior art (see for example points 1.2 and 1.3 above).

2.2[b] For examination purpose, the expression "purely aqueous solvent system" has been interpreted as referring to a biphasic system consisting of an aqueous solution and the organic substrate, which is in an amount exceeding its solubility

limit in the aqueous solution (so that it results in a second phase, eventually liquid). No organic liquid that does not take part to the enzymatic transformation as reactant or product and is not completely soluble in the aqueous solution is to be present. Particularly, no water-immiscible solvent providing a substrate reservoir and/or product extraction is considered as being involved in the claimed reaction system.

3. NOVELTY (Art. 33(2) PCT).

3.1 In view of the relevant lack of clarity (see points 2.1 and 2.2 above), the definition of the claims have been interpreted as indicated in points 2.1$^{b/c}$ and 2.2^{b} above. It has been already observed that the claimed subject-matter would not be novel, if no limiting feature is considered for the unclear definitions (see points 2.1^{a} and 2.2^{a} above).

3.1^{a} Despite the narrower interpretation of the claims, the subject-matter of claim 1 lacks novelty over D1 and D2.

3.1^{b} The emulsion membrane reactor of D1 is considered to fall within the scope of the claim because it comprises the cofactor-dependent enzymatic transformation of the ketone substrate and the enzymatic regeneration of the cofactor in an aqueous reaction medium, which is in contact with the water-immiscible fraction of the pure substrate (see point 1.1 above). The overall substrate content is clearly above the claimed limit of 50 mMol per litre of water. Moreover, the claim is silent with respect to the possibility of having filtrating membranes.

3.1^{c} D2 discloses the enzymatic reduction of 2-Heptanone by alcohol dehydrogenase in the presence of a co-substrate in an aqueous medium: this corresponds to a coupled enzymatic system comprising a cofactor-dependent enzymatic transformation and an enzymatic regeneration of the cofactor in an aqueous medium. In particular, this reaction system comprises the ketone substrate at the overall concentration of 50 mM, which is above the solubility limit of the ketone in the aqueous solution (see point 1.2 above). Hence, this system falls within the scope of claim 1.

INVENTIVE STEP (Art. 33(3) PCT).

3.2 Provided that novelty could be acknowledged, D1, D2 and D4 are independently considered to represent the relevant state of the art because they disclose coupled reactions systems of cofactor-dependent enzymes in aqueous reaction media, which do not contain any additional organic solvent or surfactant.
The subject-matter of claim 1 merely differs in the higher overall amount of the substrate.

3.2[a] The problem to be solved can be considered as the provision of an alternative coupled enzymatic system of cofactor-dependent enzymes in aqueous reaction media for the transformation of organic substrates, e.g. for biosynthetic purposes.

3.2[b] In the application (see for example the last paragraph of page 5), it is indicated that an inventive step should be acknowledged to the claimed subject-matter because it overcomes the prejudice that the presence of an organic phase, which is specifically represented by the immiscible substrate fraction, is deleterious to the enzymatic system and is therefore to be avoided.

3.2[c] It appears that such a prejudice has already been overcome in the prior art, and D2-D5 are examples of coupled enzymatic systems, which works in the presence of an organic phase (see points 1.2 and 1.3 above).

3.2[d] The skilled person would therefore have attempted to increase the overall substrate content above its water-solubility limit, and to remove the eventual filtrating membrane, in any of the enzymatic systems of D1, D2 and D4 in order to solve the problem posed, thereby obtaining a system according to claim 1. Being not limited to any specific enzyme or class of enzymes, nor to a specific substrate (see the broad scope of claim 1), the skilled person would have considered to have reasonable chances to succeed in solving the problem posed by means of an enzymatic system containing a large amount of substrate (so that an immiscible fraction of the substrate forms an organic phase in direct contact with the biological catalysts).

3.2[e] Hence, the subject-matter of claim does not involve any inventive step over any of D1, D2 and D4, taken in combination with the teaching of D2-D5 (see point 3.2[c] above).

3.3 Dependent claims 2-10 and claims 11-14 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, given the disclosure of the prior art.

3.3[a] In particular, D1-D5 relates to the use of coupled enzymatic reaction systems of cofactor-dependent enzymes for the preparation of optically active organic compounds, starting from precursor organic compounds (see points 1.1-1.3 above).

3.3[b] In addition, D6 and D7 discloses analytical and diagnostic uses of coupled enzymatic reaction systems of cofactor-dependent enzymes (see point 1.6 above).

4. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

4.1 Claims 1-14, in so far as claim 13 does not concern diagnosis and analysis, relate to enzymatic systems and their uses in the preparation of chemical compounds. Said enzymatic systems and uses can be made and applied for chemical synthesis, hence they are to be considered industrially applicable according to article 33(4) PCT.

Re Item VIII.

5. CLARITY (Art. 6 PCT).
(Further observations. See also paragraph 2 above)

5.1 Although claims 11 and 13 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only in respect of the terminology used (e.g. the use for the enzymatic transformation of organic compounds of claim 13 includes processes according to claim 11). The aforementioned claims therefore lack conciseness and therefore do not meet the requirements of Article 6 PCT.

5.2 The device described in the application on page 8 (see lines 11-15) does not fall within the scope of the claims. It is nevertheless referred to as relating to the invention. This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear, Article 6 PCT.